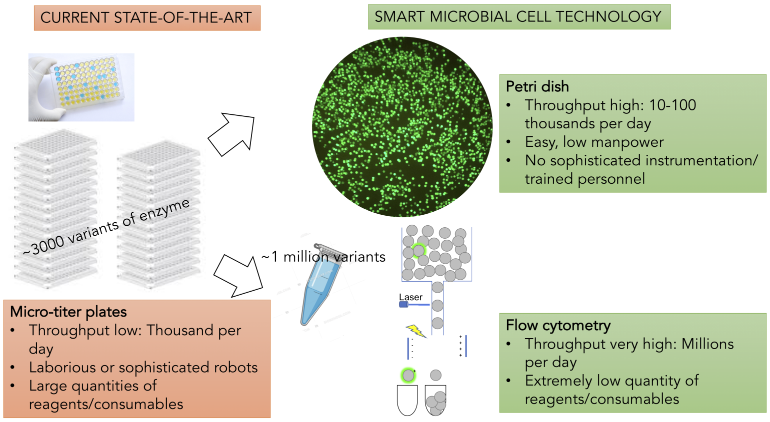
LA-UR-20-20190

Title: SMART MICROBIAL CELL TECHNOLOGY

Author(s): Jha, Ramesh Kumar

Intended for: Web

Issued: 2020-01-08



Los Alamos NATIONAL LABORATORY EST. 1943

Tech Snapshot

Biology

Published: Jan 7, 2020

SMART MICROBIAL CELL TECHNOLOGY

A high throughput screening platform for enzymes and whole cell biocatalysts

SUMMARY

Researchers at Los Alamos National Lab have developed a high throughput enzyme and whole cell biocatalyst screening platform to efficiently navigate through large sequence space. Current technologies allow building of large DNA libraries (diversity > 10^7), but current low throughput methods make it possible to test only a minute fraction (<0.1%). This disruptive technology allows rapid screening of the large library to increase the chances of capturing rare gain-of-function events that result in improved enzymes and biocatalysts.

MARKET

The enzyme market is approximately $7 billion per year. The world enzyme demand by market size (2017) is as follows: • Food and Beverage: 27% • Animal Feed: 8% • Diagnostics: 9% • Cleaning products: 18% • Biofuel: 8% • Research and Biotechnology: 17% • Other industrial: 8% • Other Specialty: 5% Furthermore, there is a large market of whole cell biocatalysts with applications in biomanufacturing and diagnostics. Our technology not only allows optimization of enzymes and whole cell biocatalysts but can also aid in discovery of new enzymes and transporters.

BENEFITS

Enzymes and whole cell biocatalysts are indispensable for multiple areas such as diagnostics, food and beverage and research and biotechnology. Biocatalysts can provide a sustainable route for producing replacement chemicals, fuels and industrial precursors. Our technology helps optimize the screening for biocatalysts to enhance throughput, reduce the use of reagents and manpower, and make the processes more economical. Moreover, our technology enables improvements in biocatalysts for stability, reduced product inhibition and catalytic efficiency.

- Enhanced throughput, screening, and catalytic efficiency
- Orders of magnitude reduction in the amount of reagents used
- Significant cost reduction in manpower needed
- Suitable for resource constrained environment (in the absence of a flow cytomter, it can be performed on a petri dish)
- Direct enzyme activity measurement

CONTACT

Marc Witkowski
witk@lanl.gov
505-665-8315

WHY WE ARE BUILDING SMART MICROBIAL CELL TECHNOLOGY

The current bottleneck in biocatalyst discovery, engineering and evolution is the absence of any high throughput screening method to scan through genetic sequence space in an expedited manner. In order to enhance the throughput, very sophisticated instruments including liquid handling robots might be required and a need of high quantity of reagents, consumables and manpower is inevitable. LANL's breakthrough technology alleviates the bottleneck in several ways. -100- to 1000-fold enhancement in throughput over the micro-titer plates method -In a micro-titer plate, single test of enzyme needs ~100 uL of total reagents and hence 10 reactions in 1 mL. The current technology, if coupled to a flow cytometer, allows a million variants to be tested in 1 mL of reagents. This is a staggering 10^5-fold lower quantity of reagents. -Minimal efforts especially due to simplicity and high throughput -Enzyme screening possible via visualization of colonies on a dish

WHAT'S BEHIND OUR TECHNOLOGY

The technology uses a custom-made sensor-reporter gene circuit. Based on the activity of the enzyme and the product formed, the sensor-reporter circuit becomes activated to give a correlated fluorescence response, which can be visualized on a petri dish under blue light and yellow filter. The technology when coupled to a flow cytometer can quantify the fluorescence and sort the cells based on the fluorescence at single cell level. A million variants of enzyme can be screened in a single sitting.

OUR COMPETITIVE ADVANTAGES

The technology can be used for a single enzyme, or a pathway, or optimization of a microbial strain. Advantages include: • Direct enzyme activity measurement not via a surrogate • Rapid way to optimize a sensor-reporter for non-model organisms • Computational design of sensors for non-natural molecules • Tunable sensors to detect a wide range of concentrations of enzyme product • Positive feedback circuit (enzyme activity feeds its own expression) to enhance the detection sensitivity • Ultra-high throughput screening efficiency of flow cytometry • Non repetitive pipetting minimizing strain injury

OUR TECHNOLOGY STATUS

• Multiple successful examples of enzyme optimization demonstrating improved expression/stability, reduced product inhibition, and enhanced catalytic efficiency • Adaptive laboratory evolution of a production strain and screening for an optimal productivity made possible We are seeking a commercialization partner to either collaborate with us through a Cooperative Research and Development Agreement (CRADA) to further develop the technology for commercial purposes or license the technology directly from us. We are very interested in the continued research and development of custom biosensors to engineer/evolve biocatalysts for gain-of-function.

PUBLICATIONS AND IP

United States Application Number 16/226,474 “Modified biosensors and biocatalysts and methods of use.”

Several publications in peer-reviewed journals published in last few years. A few currently in review:

1. Jha RK, Strauss CEM. Smart microbial cells couple catalysis and sensing to provide high throughput enzyme selection. *Manuscript under review.*

2. Bentley GJ, Narayanan N, Jha RK, Salvachua D, Elmore JR, Peabody GL, … Guss AM, Dale T, Johnson CW, Beckham GT. Engineering glucose metabolism for enhanced muconic acid production in *Pseudomonas putida* KT2440*. Manuscript accepted in Metabolic Engineering*

3. Jha RK, Narayanan N, Pandey N, Bingen J, Kern TL, Johnson CW, Strauss CEM, Beckham GT, Hennelly SP, Dale T. Sensor-enabled alleviation of product inhibition in chorismate pyruvate-lyase. ACS *Synthetic Biology* (2019), 8(4), 775-786.

4. Jha RK, Bingen JM, Johnson CW, Kern TL, Khanna P, Trettel D, Strauss CEM, Beckham GT, Dale T. A protocatechuate biosensor for *Pseudomonas putida* KT2440 via promoter and protein evolution. *Metabolic Engineering Communications* (2018), 6, 33-38.

5. Harrington L, Jha RK, Kern TL, Schmidt EN, Canales GM, Finney KB, Koppisch AT, Strauss CE and Fox DT. Rapid thermostabilization of *Bacillus thuringiensis* serovar konkukian 97-27 dehydroshikimate dehydratase through a structure-based enzyme design and whole cell activity assay. *ACS Synthetic Biology* (2016), 6(1), 120-129.

6. Jha RK, Kern TL, Kim Y, Tesar C, Jedrzejczak R, Joachimiak A, Strauss CEM. A microbial sensor for organophosphate hydrolysis exploiting an engineered specificity switch in a transcription factor. *Nucleic Acids Research* (2016), 44(17), 8490-8500.

7. Jha RK, Chakraborti S, Kern TL, Fox DT, Strauss CEM. Rosetta comparative modeling for library design: Engineering alternative inducer specificity in a transcription factor. *Proteins: Structure, Function and Bioinformatics* (2015), 83(7), 1327-40.

8. Jha RK, Kern TL, Fox DT, Strauss CEM. Engineering an Acinetobacter regulon for biosensing and high-throughput enzyme screening in *E. coli* via flow cytometry. *Nucleic Acids Research* (2014), 42(12), 8150-8160.